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# Analysis of the CAG Repeats in the SCA1 and B37 Genes in Schizophrenic and Bipolar I Disorder Patients: Tentative Association Between B37 and Schizophrenia

**Deborah J. Morris-Rosendahl,[1]* Edgar Burgert,[1] Gökhan Uyanik,[1] Antonia Mayerova,[1] Fabrice Duval,[2] Jean-Paul Macher,[2] and Marc-Antoine Crocq[2]**

*[1]Institut für Humangenetik und Anthropologie, Universität Freiburg, Freiburg, Germany*
*[2]Centre Hospitalier, Rouffach, France*

**We have genotyped unrelated French Alsatian schizophrenic and bipolar I disorder (BPD) patients and matched controls for the polymorphic CAG repeats within the genes for spinocerebellar ataxia type 1 (SCA1) and dentatorubral-pallidoluysian atrophy (B37), in order to test their possible involvement in these disorders. No alleles with abnormally expanded repeats were found in either gene in patients and controls. Differences in allele and genotype frequencies for the SCA1 CAG repeat between patients and controls were not significant, thus providing no support for its role as a possible positional candidate gene for schizophrenia and BPD in our patients. Chi square testing revealed a significant result ($P$ = 0.019) for an association between the B37 CAG repeat on chromosome 12p and schizophrenia. This result was more significant when only schizophrenics with a positive family history were compared with controls ($P$ = 0.0001). The frequencies of alleles with 14, 12, and 15 CAG repeats differed the most, respectively, between schizophrenics and controls. When choosing the median of the B37 allele distribution (15 CAG repeats) as a threshold, there were significantly more controls than schizophrenics in the group with longer alleles (15 or more repeats) and more schizophrenics with shorter alleles ($P$ = 0.002 by Fisher exact test). No particular genotype was associated with schizophrenia. This result possibly indicates linkage disequilibrium with another locus on chromosome 12p and therefore deserves further attention. No association was found between the B37 CAG repeat and patients with BPD. Am. J. Med. Genet. 74:324–330, 1997.** © 1997 Wiley-Liss, Inc.

**KEY WORDS: trinucleotide repeats; psychiatric disorders**

## INTRODUCTION

The abnormal expansion of trinucleotide repeats in the coding and regulatory regions of genes has been found to be responsible for a number of neurological disorders [reviewed by Müller et al., 1994] and genes containing trinucleotide repeats have been recognised as possible candidates for neuropsychiatric disorders [Ross et al., 1993]. Preliminary results using the repeat expansion detection (RED) method [Schalling et al., 1993], indicated a shift towards larger alleles in schizophrenia and bipolar I disorder (BPD) patients [Morris et al., 1995; O'Donovan et al., 1995]. Extended CAG repeats encoding long glutamine tracts have been found to cause Huntington's Disease [HD, The Huntington's Disease Collaborative Research Group, 1993], spinobulbar muscular atrophy [SBMA, La Spada et al., 1991], dentatorubral-pallidoluysian atrophy [DRPLA, Koide et al., 1994; Nagafuchi et al., 1994], and Haw River Syndrome [HRS, Burke et al., 1994a], and spinocerebellar ataxia types 1 [SCA1, Orr et al., 1993], 2 [SCA2, Pulst et al., 1996; Sanpei et al., 1996; Imbert et al., 1996], 3 [SCA3, and Machado-Joseph Disease, MJD, Kawaguchi et al., 1994] and probably 7 [SCA7, Trottier et al., 1995]. These are all neurodegenerative disorders, which commonly show chorea, ataxia, dementia, and sometimes psychoses [Willems, 1994] as well as the phenomenon of anticipation. Although controversial, there is evidence suggesting that the inheritance pattern of familial cases of schizophrenia and bipolar disorder (BPD) may be consistent with anticipation [Bassett and Honer, 1994; McInnes et al., 1993].

*Correspondence to: Dr. D.J. Morris-Rosendahl, Institut für Humangenetik und Anthropologie, Universität Freiburg, Breisacherstrasse 33, D-79106 Freiburg, Germany. Email: morrisro @sun2.ruf.uni-freiburg.de

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More importantly, patients with HD, DRPLA [Koide et al., 1994], a form of spinocerellar ataxia [Keddie, 1969] and hereditary cerebellar ataxia [Piqueras et al., 1995] have been reported to show psychotic symptoms, thus raising the possibility that mutations in these genes may also play a role in schizophrenia and BPD. CAG repeats in the Huntington's Disease gene, *IT-15,* have been investigated in schizophrenia patients [Rubinsztein et al., 1994a] and showed no causal effect.

The SCA1 gene is on chromosome 6p22-p23 [Banfi et al., 1993] in what was recently identified as one of the most promising regions of the human genome to harbour a schizophrenia susceptibility locus. This was revealed by independent groups performing genome-wide searches for linkage to schizophrenia in large numbers of families [Moises et al., 1995; Schwab et al., 1995; Straub et al., 1995; Wang et al., 1995]. The region of chromosome 6p highlighted by these groups is extremely large (possibly 40 cM) and probably contains a large number of genes; however, the most positive lod scores were obtained in the region extending from D6S296 to D6S285, which encompasses approximately 20 cM according to Dib et al. [1996]. The positive linkage findings could not be replicated by other workers [Gurling et al., 1995; Mowry et al., 1995] who tested these and additional markers on chromosome 6p24-22 in other families from various ethnic groups. Chromosome 6p has also been highlighted as possibly harbouring a susceptibility locus for BPD [Ginns et al., 1996]. Since the two markers D6S89 and D6S274 that flank the SCA1 gene [Banfi et al., 1993] are in the middle of the regions that showed positive lod scores for linkage to schizophrenia and BPD, we presumed that the SCA1 gene posed as a possible positional candidate for schizophrenia and BPD. Furthermore, while this manuscript was under review, Wang et al. [1996] reported a strong association of SCA1 alleles with schizophrenia in multiplex families.

SCA1 is a dominantly inherited neurodegenerative disorder characterized by ataxia, ophthalmoparesis, and variable degrees of muscle weakness, caused by progressive neuronal loss in the cerebellum, brain stem, and spinocerebellar tracts [Orr et al., 1993]. The mutation responsible for SCA1 is the expansion of a CAG repeat, which is highly polymorphic and typically contains 6-44 repeat units on normal chromosomes. SCA1 patients have one allele in the normal size range and an expanded allele that contains 40–82 repeats [Quan et al., 1995].

The expanded CAG repeat that causes DRPLA is within the B37 gene on chromosome 12p. Alleles with 7 to 23 repeats have typically been observed in control persons, whereas patients have between 49 and 75 repeats [Nagafuchi et al., 1994]. Various groups have looked for expanded CAG repeats in the B37 gene in schizophrenic patients [Lesch et al., 1994, Rubinsztein et al., 1994b, Jones Brando et al., 1996] and have only found alleles within the normal range. In addition to the search for expanded alleles, Guedj et al. [1996] tested for an association with schizophrenia using the transmission disequilibrium test, and found no association in their families. However Sasaki et al. [1996] found a trend towards a difference in allele counts between their sample of unrelated schizophrenic patients and controls. Only one group has tested BPD patients at the DRPLA locus [Jain et al., 1996] and found no evidence of expanded alleles in a sample of 24 patients. We wished to investigate the DRPLA locus further by an association study in larger samples of schizophrenic and BPD patients and controls. In this study we have genotyped samples from schizophrenic and BPD patients and control subjects for CAG repeats in the B37 and SCA1 genes in order to look for alleles which may extend into the pathological range in patients and to perform association studies to detect possible linkage disequilibrium on chromosome 6p due to the proximity to another predisposing locus.

## METHODS

### Patient and Control Samples

The study was performed after approval by the local Ethics Committee and with the informed consent of the patients. All subjects were unrelated and were of French or Alsatian ancestry; Mediterranean Caucasians, Asians, and Africans were excluded. Patients meeting DSM-IV criteria [American Psychiatric Association, 1994] for schizophrenia or bipolar I disorder were recruited among subjects hospitalised in a single hospital in Alsace. Diagnoses were established according to DSM-IV criteria prior to genetic analysis. Patients were examined with an unstructured clinical interview (by M.-A.C.) and also with a structured clinical interview [Schedule for Affective Disorders and Schizophrenia-Lifetime Version, Spitzer et al., 1975] conducted by another psychiatrist (F.D.). Final diagnoses were made by a consensus of two psychiatrists and patients were not included in the study in cases of disagreement. Family history was assessed by interviewing patients and their relatives, and from case records. Patients were regarded as having a positive family history (FH+) if they had a first degree relative who had been diagnosed as having either schizophrenia or BPD; more distant relatives were not considered since it was not feasible to gather this information in a consistent and reliable manner. Conversely, patients were classified as having a negative family history (FH–) if no first degree relatives were affected with a psychiatric disorder. Family history status could not be ascertained for all patients. Controls were recruited from among healthy staff and students at the same hospital, were all older than 25 years and had no history of psychiatric disease. The number of patients and controls tested at each locus, the mean age and numbers of females and males in each of the sample groups are given in Table I. The average age of the BPD patient group was significantly greater than those of both the control and schizophrenic groups, due to the typical later age of onset and diagnosis of the illness.

### Genotyping

High molecular weight DNA was extracted from EDTA blood obtained after informed consent. All genotyping was performed "blindly," with the clinical status of the samples unknown to the genotyper. PCR ampli-

TABLE I. Samples Analysed for the SCA1 and B37 CAG Repeats Together With the Mean Age of Individuals and Numbers of Females and Males Within Each Group*

| Group | Number of individuals | FH+ | FH– | Mean age ± SD | Females/males |
|---|---|---|---|---|---|
| SCA1 | | | | | |
| Schizophrenia | 76 | 22 | 26 | 40.8 ± 11.6 | 21/55 |
| BPD | 35 | 17 | 5 | 56.4 ± 15.2 | 15/20 |
| Controls | 73 | — | — | 37.6 ± 9.1 | 40/33 |
| B37 | | | | | |
| Schizophrenia | 83 | 23 | 23 | 40.5 ± 11.7 | 23/60 |
| BPD | 33 | 17 | 5 | 53.8 ± 12.6 | 14/19 |
| Controls | 65 | — | — | 39.8 ± 14.6 | 36/29 |

*FH+, positive family history; FH–, negative family history.

fication was performed using primers reported by Müller et al. [1994] for the SCA1 CAG repeat and by Li et al. [1993] for the B37 CAG repeat, after 5′ end-labelling of the forward primer with gamma $^{32}$P-ATP using T4 polynucleotide kinase. PCR conditions were as follows, for SCA1 CAG: 30 cycles of 95°C/1′, 54°C/1′, 72°C/2′; for B37 CAG: 10 cycles of 95°C/1′, 65°C/1′30″, 72°C/2′, followed by 20 cycles with the annealing temperature reduced to 64°C. For B37, 5% dimethylsulphoxide (DMSO) and Taq Extender (Stratagene, La Jolla, CA) were added to the reaction mix. All reactions were preceded by denaturation at 95°C for 5′ and followed by a final extension at 72°C for 7′. PCR products were analysed on 6% denaturing polyacrylamide gels, followed by autoradioagraphy. M13 sequence was used to determine the sizes of alleles in previously diagnosed DRPLA and SCA1 patient samples containing expanded CAG repeat alleles. These patients' samples, together with additional samples from reference individuals, were run in parallel with the samples on all gels to allow gel-to-gel standardisation.

### Statistical Analysis

The chi square ($\chi^2$) test was performed to determine whether the genotypes at each locus were in Hardy-Weinberg equilibrium in each sample group; as well as to detect differences in allele and genotype frequencies and the proportion of homozygotes, between the patient and control groups. For all $\chi^2$ testing, cells containing expected values of less the 5 were pooled, with rare alleles being pooled with the closest adjacent allele of a larger size.

Since alleles containing trinucleotide repeats can be ranked according to their size, and they are not necessarily normally distributed, the non-parametric Mann-Whitney U Test of rank was performed to explore whether a group had significantly longer alleles. The Fisher exact test was used for two-way contingency tables. The Bonferroni correction was applied to correct for multiple comparisons [Overall and Rhoades, 1987].

## RESULTS

The number of CAG repeats in the SCA1 and B37 genes were determined in schizophrenic and BPD patients and controls (Tables II and III). The distributions of SCA1 and B37 alleles, as represented by allele frequencies, are shown in Figure 1 (A and B, respectively). Genotype frequencies at the SCA1 and B37 loci were in Hardy-Weinberg equilibrium in each sample group (B37: $P>0.652$ in each group, SCA1: $P>0.717$ in each group). No expanded CAG repeat alleles in the pathological range for each of these genes were observed.

The chi square and Mann-Whitney U tests were performed to evaluate the differences in allele and genotype frequencies between the schizophrenic and BPD patients and controls (Table IV). No significant differences were observed in allele or genotype frequencies for the CAG repeat in the SCA1 gene between schizophrenic patients and controls, nor between BPD patients and controls. Since a recent report of Wang et al. [1996] shows a significant association between the SCA1 CAG repeat and schizophrenia in multiplex families, we investigated possible differences in allele frequencies between our schizophrenic patients with and without a positive family history. We found no significant difference between FH+ and FH– patients ($\chi^2 = 0.774$, 2 df, $P = 0.679$), nor between FH+ patients and controls ($\chi^2 = 0.210$, 2 df, $P = 0.546$) or FH– patients and controls ($\chi^2 = 0.171$, 2 df, $P = 0.918$).

TABLE II. Number of Chromosomes Observed With Each of the SCA1 Alleles in Schizophrenic and Bipolar Disorder Patients, and Controls

| SCA1 allele (number of CAG repeats) | Schizophrenic | Control | BPD |
|---|---|---|---|
| 23 | 1 | 0 | 0 |
| 24 | 0 | 0 | 0 |
| 25 | 0 | 0 | 0 |
| 26 | 0 | 0 | 0 |
| 27 | 1 | 1 | 0 |
| 28 | 14 | 9 | 2 |
| 29 | 52 | 53 | 22 |
| 30 | 45 | 49 | 26 |
| 31 | 20 | 19 | 7 |
| 32 | 6 | 11 | 9 |
| 33 | 8 | 2 | 1 |
| 34 | 2 | 0 | 0 |
| 35 | 1 | 1 | 2 |
| 36 | 2 | 0 | 1 |
| 37 | 0 | 1 | 0 |
| | n = 152 | n = 146 | n = 70 |

TABLE III. Number of Chromosomes Observed With Each of the B37 Alleles in Schizophrenic and BPD Patients, and Controls

| B37 allele (number of CAG repeats) | Schizophrenic | Control | BPD |
|---|---|---|---|
| 7 | 1 | 0 | 0 |
| 8 | 10 | 7 | 3 |
| 9 | 10 | 4 | 3 |
| 10 | 9 | 6 | 5 |
| 11 | 8 | 8 | 2 |
| 12 | 10 | 1 | 1 |
| 13 | 4 | 3 | 1 |
| 14 | 19 | 4 | 6 |
| 15 | 26 | 34 | 19 |
| 16 | 36 | 36 | 16 |
| 17 | 18 | 13 | 5 |
| 18 | 11 | 5 | 4 |
| 19 | 2 | 5 | 1 |
| 20 | 2 | 2 | 0 |
| 21 | 0 | 1 | 0 |
| 22 | 0 | 0 | 0 |
| 23 | 0 | 0 | 0 |
| 24 | 0 | 0 | 0 |
| 25 | 0 | 0 | 0 |
| 26 | 0 | 0 | 0 |
| 27 | 0 | 0 | 0 |
| 28 | 0 | 1 | 0 |
| | n = 166 | n = 130 | n = 66 |

## B37 CAG Repeat in Schizophrenic and BPD Patients and Controls

As previously reported for Caucasians [Watkins et al., 1995], the distribution of alleles at the B37 gene is bimodal, with peaks around alleles 8–11 and 16 CAG repeats. There were no significant differences between BPD patients and controls for alleles of the B37 CAG repeat. A mildly significant difference was observed between the allele frequencies of schizophrenics and controls using the chi square test ($\chi^2 = 21.38$, 10 df, $P = 0.019$). However, applying the Bonferroni correction yielded a $P$ value of 0.076, if taken into account that we performed four independent comparisons to test two disorders at two loci. The largest relative differences between observed and expected values, and thus the largest contributions to the chi square, were observed, in order of importance, for alleles with $(CAG)_{14}$, $(CAG)_{12}$, and $(CAG)_{15}$. Controls were over represented at allele $(CAG)_{15}$, which was also the median of the allelic distribution, whereas schizophrenics were more often represented at the two frequent alleles preceding the median (alleles $(CAG)_{12}$ and $(CAG)_{14}$). Thus when choosing the median of the B37 allele distribution (15 CAG repeats) as a threshold, there were significantly more controls than schizophrenics in the group with longer alleles (15 or more repeats) and more schizophrenics with shorter alleles ($P = 0.002$ by Fisher exact test). Using the Mann-Whitney U test of rank, the difference in allele counts between schizophrenics and controls was not significant, although the $P$ value ($P = 0.079$) may indicate a trend towards significance.

The difference in allele frequencies between schizophrenic patients and controls appeared to be influenced by family history. Allele frequencies did not differ significantly between FH– and FH+ patients ($\chi^2 = 8.44$, 4 df, $P = 0.077$) nor between FH– patients and controls ($\chi^2 = 1.50$, 4 df, $P = 0.827$). However there was a significant difference between FH+ patients and controls ($\chi^2 = 22.67$, 4 df, $P = 0.0001$), which remains significant after Bonferroni correction. When choosing the median of 15 CAG repeats as a threshold, as above, there were significantly more FH+ schizophrenics than controls in the group with shorter alleles (<15 repeats) ($P = 0.0005$, Fisher exact test), whereas there was no significant difference between FH– schizophrenics and controls ($P = 0.343$, Fisher exact test).

Gender differences in the course and presentation of schizophrenia are well-known and are summarised in DSMIV [American Psychiatric Association, 1994]. We therefore hypothesized that dividing our sample by gender might increase the homogeneity of the sample. Patient gender appeared not to affect the significance of the difference in B37 alleles between patients and controls. The difference between male schizophrenics and all controls ($\chi^2 = 17.99$, 10 df, $P = 0.055$) was more significant than that between female schizophrenics and all controls ($\chi^2 = 4.93$, 4 df, $P = 0.295$), however, this is probably due to the larger number of male patients available for comparison. There were no significant differences between males and females within the patient or control groups alone.

There were no significant differences in genotype frequencies between patients and controls as determined by the chi square test. Moreover, the proportion of homozygotes at the B37 locus (26.2% in controls, 15.7% in schizophrenics, and 30.3% in BPD patients) did not dif-

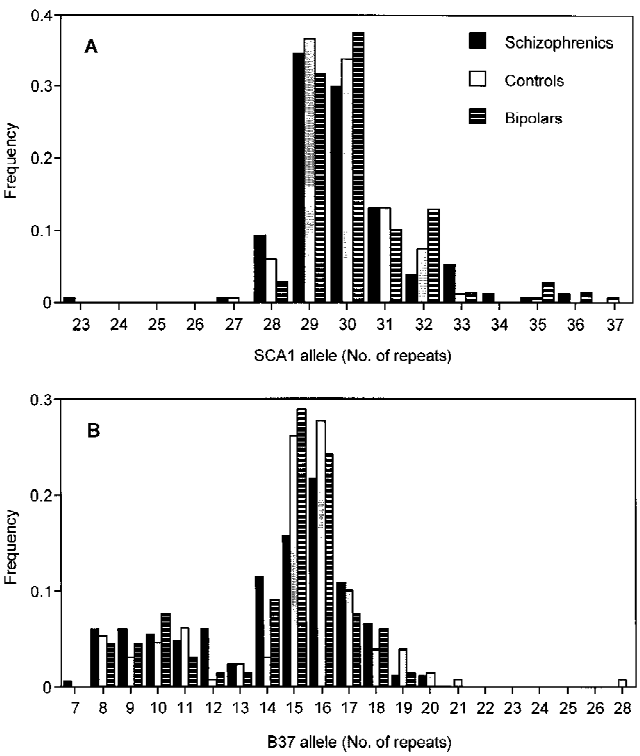


Fig. 1. Allele frequencies of the CAG repeats in the SCA1 (**A**) and B37 (**B**) genes in schizophrenic and bipolar I disorder patients and controls.

TABLE IV. Statistical Testing of the Differences in Allele Counts, Allele Size Rank (Mann-Whitney U Test), and Genotype Frequencies Between Schizophrenic Patients and Controls and Between BPD Patients and Controls at the SCA1 and B37 Loci

| | Schizophrenia vs. control | BPD vs. control |
|---|---|---|
| SCA1 | | |
| Allele counts | $\chi_5^2 = 7.71$, $P = 0.173$ | $\chi_3^2 = 3.97$, $P = 0.265$ |
| Allele size (M-W U test) | $P = 0.930$ | $P = 0.140$ |
| Genotype frequencies | $\chi_6^2 = 2.96$, $P = 0.813$ | $\chi_3^2 = 4.64$, $P = 0.200$ |
| B37 | | |
| Allele counts | $\chi_{10}^2 = 21.38$, $P = 0.019$ | $\chi_6^2 = 2.98$, $P = 0.811$ |
| Allele size (M-W U test) | $P = 0.079$ | $P = 0.234$ |
| Genotype frequencies | $\chi_7^2 = 6.46$, $P = 0.487$ | $\chi_4^2 = 2.98$, $P = 0.562$ |

fer significantly between diagnostic groups ($\chi^2 = 3.91$, 2 df, $P = 0.142$).

## DISCUSSION

The use of linkage analysis for gene mapping, which has proved so successful for monogenic disorders, has limited power to detect genes of modest effect [Risch and Merikangas, 1996], such as many of those likely to play a role in multifactorial psychiatric disorders. At most, one can hope to highlight a chromosomal region where such a gene may reside. This has been the case for schizophrenia and BPD, with the elucidation of chromosome 6p as such a promising area. Since only a small and possibly unknown number of families will be linked to the locus tested, the detection of linkage disequilibrium by haplotype analysis, as a means of narrowing down the candidate gene region, is difficult. Possibly a more effective way of doing this is to conduct follow-up association studies in additional patient and control samples, by using polymorphic markers, optimally within candidate genes, in the region showing linkage.

As discussed by other authors [Ross et al., 1993; Petronis and Kennedy, 1995], we believe that genes containing trinucleotide repeats may play a role in schizophrenia and BPD. Considering the recent positive linkage results for schizophrenia and BPD on chromosome 6p, we assumed that the SCA1 gene was a good positional candidate for both disorders. Our finding of alleles only within the normal range in the SCA1 gene in all the patients tested excludes the possibility of expanded CAG repeats being pathological in our sample of schizophrenic and BPD patients. Moreover, the absence of any significant differences in allele or genotype frequencies for the SCA1 CAG repeat, between our patient groups and controls, indicates that it is unlikely that the SCA1 gene plays an important role or is very close to another gene of major effect in the patients included in this study. The distributions of SCA1 and B37 alleles in our control samples were very similar to those published by Watkins et al. [1995] who studied the allele frequencies at various trinucleotide repeat loci in different populations, and whose group of Caucasians comprised American males of Northern European ancestry and French individuals. It is unlikely that expanded trinucleotide alleles were missed in our patient samples, since our PCR conditions have been optimised to amplify large alleles and patients with DRPLA and SCA1 with alleles of 64 and 57 repeats, respectively, were included in all experiments. Since we have not observed an excess of homozygotes in any of the sample groups, it is not likely that allelic dropout need be considered as influential.

Wang et al. [1996] reported a strong association of SCA1 alleles with schizophrenia in multiplex families. It is not yet clear whether these results, obtained using the transmission disequilibrium test (TDT), reflect linkage in the vicinity of the SCA1 locus or whether the SCA1 gene itself may affect the risk of schizophrenia in their patients. It is possible that the results of Wang et al. [1996] reflect an association observed only in patients with a strong family history. We found no significant difference in allele frequencies between our patients with and without a positive family history of schizophrenia at the SCA1 locus. Wang et al. [1996] also observed a 41-repeat allele in an affected subject, although the segregation of this allele in the patient's family is not clear.

Clinically, the B37 gene poses as a better candidate for schizophrenia than the SCA1 gene, because of the schizophrenic symptoms shown by some patients with DRPLA [Koide et al., 1994]. The slightly significant difference in allele frequencies between our schizophrenic patients and controls is interesting. However, the difference in allele frequencies between all patients and controls was not significant after the Bonferroni correction of the chi square result. The difference also did not reach significance using the Mann-Whitney U test of rank and therefore deserves some caution. We postulated that the latter test may be more appropriate than the chi square test for this analysis, since the alleles at trinucleotide repeat loci are sequential. Alleles can therefore be assigned a rank, according to their repeat number and our prior knowledge of the pathological effects of larger alleles in neurological disorders.

Despite the small sample sizes, the increased significance of the difference in allele frequencies between patients with a positive family history in first degree relatives, and controls, indicates that it would be worthwhile testing this locus in families. A number of groups have conducted studies at the B37 locus using familial cases of schizophrenia and no statistically significant association has been found; although, with the exception of the study by Guedj et al. [1996], not all results have been conclusive. Using the transmission

disequilibrium test in 44 American families, Guedj et al. [1996] found no association between the B37 CAG repeat and schizophrenia.

Although no statistically significant association between the B37 gene and schizophrenia has been found in other studies, some nonsignificant trends have been apparent. Lesch et al. [1994] found no significant differences in mean allele size between familial schizophrenic patients with periodic catatonia and controls, but the modal repeat length in schizophrenics was one repeat less (14) than that in controls (15). The allele distributions, shown by Lesch et al. [1994] for patients and controls, resemble our distribution in schizophrenic patients and controls very closely, with both studies showing a great excess of alleles with 14 repeats in the schizophrenic group, as opposed to controls. The patient and controls studied by Lesch et al. [1994] were all recruited from an area in West Germany, not geographically far removed from Alsace, France, which makes a comparison between the two studies valid. Rubinsztein et al. [1994b] investigated unrelated familial cases of schizophrenia from the USA, United Kingdom and Italy. Since the emphasis in their study was on the search for expanded alleles, they did not look for an association with allele and genotype frequencies. However, the distribution of B37 alleles shows an excess of alleles with 15 repeats in their group of 55 unrelated schizophrenia patients, compared with controls. This allele may well represent the allele designated by us and Lesch et al. [1994] as having 14 repeats (due to a difference in repeat number designation between studies]. The control samples used by Rubinsztein et al. [1994b] were from the Japanese population in which a different normal allele distribution from Caucasians has been observed [Burke et al., 1994b; Watkins et al., 1995], thus making a patient-control comparison problematic. Sasaki et al. [1996] who investigated North American and Italian patients and controls, found nonsignificant differences in the number of alleles with 7, 8, and 13 repeats, with many more schizophrenics having allele 13 than controls. This may also indicate an excess of alleles in the 13–15 repeat range in schizophrenic patients. However, the allele distributions shown by Sasaki et al. [1996] appear to be shifted towards smaller alleles, with no alleles with more than 16 repeats observed, which makes comparison with our data difficult.

Investigations at the Huntington's Disease [Goldberg et al., 1993; Myers et al., 1993] and B37 [Yanagisawa et al., 1996] CAG loci in different world populations have led to the hypothesis that large normal alleles in triplet-repeat disease form an important source for disease predisposition. Thus it is unlikely that a trend towards smaller alleles at the B37 locus would play a causative role in our schizophrenic patients. This does not exclude the possibility that other types of DNA variation within or flanking the B37 gene may have a functional effect that would increase the risk of schizophrenia and may be in linkage disequilibrium with the CAG repeat. We postulate however, that our result more likely indicates linkage disequilibrium with another closely linked locus on chromosome 12p. A significant positive association between a particular CD4 allele (A4) and Belgian schizophrenic patients has been reported by Ghabanbasani et al. [1994]. Since the CD4 locus is closely linked to that for DRPLA on chromosome 12p [Nagafuchi et al., 1994], it will be interesting to test the CD4 polymorphism in our patients and controls.

In this study we have shown that there is no association between the SCA1 CAG repeat and schizophrenia nor BPD in our sample of patients. There does, however appear to be a mild, but detectable association between the B37 gene and schizophrenia in our patients. It is difficult to predict whether the effect observed indicates linkage disequilibrium with another closely linked locus, or may be involved in the pathology of schizophrenia. We nevertheless feel that this result deserves further investigation, possibly in other large sample cohorts.

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## REFERENCES

American Psychiatric Association (1994): "Diagnostic and Statistical Manual of Mental Disorders," Fourth Edition. Washington DC; American Psychiatric Association.

Banfi S, Chung M-y, Kwiatkowski TJ Jr, Ranum LPW, McCall AE, Chinault AC, Orr HT, Zoghbi HY (1993): Mapping and cloning of the critical region for the spinocerebellar ataxia type 1 gene (SCA1) in a yeast artificial chromosome contig spanning 1.2 Mb. Genomics 18:627–635.

Bassett AS, Honer WG (1994): Evidence for anticipation in schizophrenia. Am J Hum Genet 54:846–870.

Burke JR, Wingfield MA, Lewis KE, Roses AD, Lee JE, Hulette C, Pericak-Vance MA, Vance JM (1994a): The Haw River Syndrome: Dentatorubropallidoluysian atrophy (DRPLA) in an African-American family. Nat Genet 7:521–524.

Burke JR, Ikeuchi T, Koide R, Tsuji S, Yamada M, Pericak-Vance MA, Vance JM (1994b): Dentatorubral-pallidoluysian atrophy and Haw River syndrome. Lancet 344:1711–1712.

Dib C, Fauré S, Fizames C, Samson D, Drouot N, Vignal A, Millasseau P, Marc S, Hazan J, Seboun E, Lathrop M, Gyapay G, Morissette J, Weissenbach J (1996): A comprehensive genetic map of the human genome based on 5,264 microsatellites. Nature 380:152–154.

Ghabanbasani Zamani M, De Hert M, Spaepen M, Hermans M, Marynen P, Cassiman J-J, Peuskens J (1994): Study of the possible association of HLA class II, CD4, and CD3 polymorphisms with schizophrenia. Am J Med Genet (Neuropsychiatr Genet) 54:372–377.

Ginns EI, Ott J, Egeland JA, Allen CR, Fann CSJ, Pauls DL, Weissenbach J, Carulli JP, Falls KM, Keith TP, Paul SM (1996): A genome-wide search for chromosomal loci linked to bipolar affective disorder in the Old Order Amish. Nat Genet 12:431–435.

Goldberg YP, Kremer B, Andrew SE, Theilmann J, Graham RK, Squitieri F, Telenius H, Adam S, Sajoo S, Starr E, Heiberg A, Wolff G, Haydn MR (1993): Molecular analysis of new mutations in Huntington's disease: Intermediate alleles and sex of origin effects. Nat Genet 5:174–179.

Guedj F, Cao Q, Cravchik A, Ram A, Badner J, Gershon ES, Gejman PV (1996): Analysis of DRPLA trinucleotide repeats in schizophrenia. Psychiatr Genet 6:33–34.

Gurling H, Kalsi G, Hui-Sui Chen, A, Green M, Butler R, Read T, Murphy P, Curtis D, Sharma T, Petursson H, Brynjolfsson J (1995): Schizophrenia susceptibility and chromosome 6p24-22. Nat Genet 11:234–235.

Imbert G, Saudou F, Yvert G, Devys D, Trottier Y, Garnier J-M, Weber C, Mandel J-L, Cancel G, Abbas N, Dürr A, Didierjean O, Stevanin G, Agid Y, Brice A (1996): Cloning of the gene for spinocerebellar ataxia 2 reveals a locus with high sensitivity to expanded CAG/glutamine repeats. Nat Genet 14:285–291.

Jain S, Leggo J, DeLisi LE, Crow TJ, Margolis RL, Li S-H, Goodburn S, Walsh C, Paykel ES, Ferguson-Smith MA, Ross CA, Rubinsztein DC (1996): Analysis of thirteen trinucleotide repeat loci as candidate genes for schizophrenia and bipolar affective disorder. Am J Med Genet (Neuropsychiatr Genet) 67:139–146.

Jones Brando L, Yolken R, Herman MM, Kleinman JE, Ross CA, Torrey EF (1996): Analysis of the DRPLA triplet repeat in brain tissue and leukocytes from schizophrenics. Psychiatr Genet 6:1–5.

Kawaguchi Y, Okamoto T, Taniwaki M, Aizawa M, Inoue M, Katayama S, Kawakami H, Nakamura S, Nishimura M, Akiguchi I, Kimura J, Narumiya S, Kakizuka A (1994): CAG expansions in a novel gene for Machado-Joseph disease at chromosome 14q32.1. Nat Genet 8:221–228.

Keddie KMG (1969): Hereditary ataxia, presumed to be of the Menzel type, complicated by paranoid psychosis, in a mother and two sons. J Neurol Neurosurg Psychiatry 32:82–87.

Koide R, Ikeuchi T, Onodera O, Tanaka H, Endo K, Takahashi H, Kondo R, Ishikawa A, Hayashi T, Saito M, Tomoda A, Miike T, Naito H, Ikuta F, Tsuji S (1994): Unstable expansion of CAG repeat in hereditary dentatorubral-pallidoluysian atrophy (DRPLA). Nat Genet 6:9–12.

La Spada AR, Wilson EM, Lubahn DB, Harding AE, Fischbeck KH (1991): Androgen receptor gene mutations in X-linked spinal and bulbar muscular atrophy. Nature 352:77–79.

Lesch KP, Stöber G, Balling U, Franzek E, Li SH, Ross CA, Newman M, Beckmann H, Riederer P (1994): Triplet repeats in clinical subtypes of schizophrenia: Variation at the DRPLA (B37 CAG repeat) locus is not associated with periodic catatonia. J Neural Transm (Gen Sect) 98: 153–157.

Li S-H, McInnis MG, Margolis RL, Antonarakis SE, Ross CA (1993): Novel triplet repeat containing genes in human brain: Cloning, expression and length polymorphisms. Genomics 16:572–579.

McInnis MG, McMahon FJ, Chase GA, Simpson SG, Ross CA, De Paulo R Jr (1993): Anticipation in bipolar affective disorder. Am J Hum Genet 53:385–390.

Moises HW, Yang L, Kristbjarnarson H, Wiese C, Byerley W, Macciardi F, Arolt V, Blackwood D, Liu X, Sjögren B, Aschauer HN, Hwu H-G, Jang K, Livesley WJ, Kennedy JL, Zoega T, Ivarsson O, Bui M-T, Yu M-h, Havsteen B, Commenges D, Weissenbach J, Schwinger E, Gottesman II, Pakstis AJ, Wetterberg L, Kidd KK, Helgason T (1995): An international two-stage genome-wide search for schizophrenia susceptibility genes. Nat Genet 11:321–324.

Morris AG, Gaitonde E, McKenna PJ, Mollon JD, Hunt DM (1995): CAG repeat expansions and schizophrenia: association with disease in females and with early age-at-onset. Hum Mol Genet 4:1957–1961.

Mowry BJ, Nancarrow DJ, Lennon DP, Sandkuijl LA, Crowe RR, Silverman JM, Mohs RC, Siever LJ, Endicott J, Sharpe L, Walters MK, Hayward NK, Levinson DF (1995): Schizophrenia susceptibility and chromosome 6p24-22. Nat Genet 11:233–234.

Müller U, Graeber MB, Haberhausen G, Köhler A (1994): Molecular basis and diagnosis of neurogenetic disorders. J Neurol Sci 124:119–140.

Myers RH, MacDonald ME, Koroshetz WJ, Duyao MP, Ambrose CM, Taylor SAM, Barnes G, Srinidhi J, Lin CS, Whaley WL, Lazzarini AM, Schwarz M, Wolff G, Bird ED, Vonsattel J-PG, Gusella JF (1993): *De novo* expansion of a $(CAG)_n$ repeat in sporadic Huntington's disease. Nat Genet 5:168–173.

Nagafuchi S, Yanagisawa H, Sato K, Shirayama T, Ohsaki E, Bundo M, Takeda T, Tadokoro K, Kondo I, Murayama N, Tanaka Y, Kikushima H, Umino K, Kurosawa H, Furukawa T, Nihei K, Inoue T, Sano A, Komure O, Takahashi M, Yoshizawa T, Kanazawa I, Yamada M (1994): Dentatorubral and pallidoluysian atrophy expansion of an unstable CAG trinucleotide on chromosome 12p. Nat Genet 6:14–18.

O'Donovan MC, Guy C, Craddock N, Murphy KC, Cardno AG, Jones LA, Owen MJ, McGuffin P (1995): Expanded CAG repeats in schizophrenia and bipolar disorder. Nat Genet 10:380–381.

Orr, HT, Chung MY, Banfi S, Kwiatkowski Jr J, Servadio A, Beaudet AL, McCall AE, Duvick LA, Ranum LPW, Zoghbi HY (1993): Expansion of an unstable trinucleotide CAG repeat in spinocerebellar ataxia type 1. Nat Genet 4:221–226.

Overall JE, Rhoades HM (1987): Adjusting p Values for Multiple Tests of Significance. In Meltzer HY (ed): "Psychopharmacology: The Third Generation of Progress." New York: Raven Press, pp 1013–1018.

Petronis A, Kennedy JL (1995): Unstable genes: Unstable mind? Am J Psychiatry 152:164–172.

Piqueras JF, Santos J, Visedo G, Pérez de Castro I, Puertollano R, Montejo J, Ramo Tello C, Valle J (1995): Familial cosegregation of manic-depressive illness and a form of hereditary cerebellar ataxia. Am J Med Genet (Neuropsychiatr Genet) 60:206–209.

Pulst S-M, Nechiporuk A, Nechiporuk T, Gispert S, Chen X-N, Lopes-Cendes I, Pearlman S, Starkman S, Orozco-Diaz G, Lunkes A, DeJong P, Rouleau GA, Auberger G, Korenberg JR, Figueroa C, Sahba S (1996) Moderate expansion of a normally biallelic trinucleotide repeat in spinocerebellar ataxia type 2. Nat Genet 14:269–276.

Quan F, Janas J, Popovich BW (1995) A novel CAG repeat configuration in the SCA1 gene: Implications for the molecular diagnostics of spinocerebellar ataxia type 1. Hum Mol Genet 4:2411–2413.

Risch N, Merikangas K (1996): The future of genetic studies of complex human diseases. Science 273:1516–1517.

Ross CA, McInnis MG, Margolis RL, Li SH (1993): Genes with triplet repeats: candidate mediators of neuropsychiatric disorders. Trends Neurosci 16:254–260.

Rubinsztein DC, Leggo J, Goodburn S, Crow TJ, Lofthouse R, DeLisi LE, Barton DE, Ferguson-Smith MA (1994a): Study of Huntington's disease (HD) gene CAG repeats in schizophrenic patients shows overlap of the normal and HD affected ranges but absence of correlation with schizophrenia. J Med Genet 31:690–693.

Rubinsztein DC, Leggo J, Goodburn S, Barton DE, Ferguson-Smith MA, Ross CA, Li SH, Lofthouse R, Crow TJ, DeLisi LE (1994b): B37 repeats are normal in most schizophrenic patients. Br J Psychiatry 164:851–852.

Sanpei K, Takano H, Igarashi S, Sato T, Oyake M, Sasaki H, Wakisaka A, Tashiro K, Ishida Y, Ikeuchi T, Koide R, Saito M, Sato A, Tanaka T, Hanyu S, Takiyama Y, Nishizawa M, Shimizu N, Nomura Y, Segawa M, Iwabuchi K, Eguchi I, Tanaka H, Takahashi H, Tsuji S (1996): Identification of the spinocerebellar ataxia type 2 gene using a direct identification of repeat expansion and cloning technique, DIRECT. Nat Genet 14:277–284.

Sasaki T, Billet E, Petronis A, Ying D, Parsons T, Macciardi FM, Meltzer HY, Lieberman J, Joffe RT, Ross CA, McInnis MG, Li S-H, Kennedy JL (1996): Psychosis and genes with trinucleotide repeat polymorphism. Hum Genet 97:244–246.

Schalling M, Hudson T, Buetow K, Houseman D (1993): Direct detection of trinucleotide repeats in the human genome. Nat Genet 4:135–139.

Schwab SG, Albus M, Hallmayer J, Hönig S, Borrmann M, Lichtermann D, Ebstein RP, Ackenheil M, Lerer B, Risch N, Maier W, Wildernauer DB (1995): Evaluation of a susceptibility gene for schizophrenia on chromosome 6p by multipoint affected sib-pair linkage analysis. Nat Genet 11:325–327.

Spitzer RL, Endicott J (1975): "Schedule for Affective Disorders and Schizophrenia, Lifetime Version." New York: Biometrics Research Division, New York State Psychiatric Institute.

Straub RE, MacLean CJ, O'Neill FA, Burke J, Murphy B, Duke F, Shinkwin R, Webb BT, Zhang J, Walsh D, Kendler KS (1995): A potential vulnerability locus for schizophrenia on chromosome 6p24-22: evidence for genetic heterogeneity. Nat Genet. 11:287–293.

The Huntington's Disease Collaborative Research Group (1993): A novel gene containing a trinucleotide repeat that is expanded and unstable on Huntington's Disease chromosomes. Cell 72:971–983.

Trottier Y, Lutz Y, Stevanin G, Imbert G, Devys D, Cancel G, Saudou F, Weber C, David G, Tora L, Agid Y, Brice A, Mandel J-L (1995): Polyglutamine expansion as a pathological epitope in Huntington's disease and four dominant cerebellar ataxias. Nature 378:403–406.

Wang S, Sun C-e, Walczak CA, Ziegle JS, Kipps BR, Goldin LR, Diehl SR (1995): Evidence for a susceptibility locus for schizophrenia on chromosome 6pter-p22. Nat Genet 10:41–46.

Wang S, Detera-Wadleigh SD, Coon H, Sun C-E, Goldin LR, Duffy DL, Byerley WF, Gershon ES, Diehl SR (1996): Evidence of linkage disequilibrium between schizophrenia and the SCA1 CAG repeat on chromosome 6p23. Am J Hum Genet 59:731–736.

Watkins WS, Bamshad M, Jorde LB (1995): Population genetics of trinucleotide repeat polymorphisms. Hum Mol Genet 4:1485–1491.

Willems P (1994): Dynamic mutations hit double figures. Nat Genet 8:213–215.

Yanagisawa H, Fujii K, Nagafuchi S, Nakahori Y, Nakagome Y, Akane A, Nakamura M, Sano A, Komure O, Kondo I, Jin DK, Sørensen SA, Potter NT, Young SR, Nakamura K, Nukina N, Nagao Y, Tadokoro K, Okuyama T, Miyashita T, Inoue T, Kanazawa I, Yamada M (1996): A unique origin and multistep process for the generation of expanded DRPLA triplet repeats. Hum Mol Genet 5:373–379.